

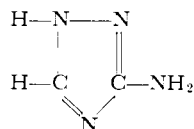
Residual Activity of 3-Amino-1,2,4-triazole in Soils

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A colorimetric method for the determination of 3-amino-1,2,4-triazole residues in soils has been developed. Investigations on the disappearance of aminotriazole in soils indicated that the compound becomes tightly adsorbed to soil particles. Not only does the chemical seem to partake in the soil's base exchange system, but it has the tendency also to complex metals. Studies on aminotriazole toxicity to tomato seedlings were correlated with chemical analyses and it was found that the biological response to plants is proportional to the amount recoverable in any soil type.

THE UNUSUAL GROWTH-REGULATING PROPERTIES of 3-amino-1,2,4-triazole, commonly called aminotriazole, have been explored during the past 3 years. It is a heterocyclic compound possessing the following structural formula:



Probably the most dramatic effect of aminotriazole on growing plants is its interference with chlorophyll formation. This property has stimulated the investigation of the compound as a herbicide, cotton defoliant, and inhibitor of second growth. The compound shows promise for commercial control of perennials such as Canada thistle (5), quackgrass (10), Johnson grass (12), nut grass (2), and woody plants like poison ivy and poison oak (7).

Aminotriazole not only produces marked biological responses to plants at very low concentrations of material but it has been shown also to be translocated throughout the growing plant (3). At sublethal dosages albinism is evident in the new growth of treated plants. Carbohydrate metabolism, respiration, and general growth responses are also affected (3).

Because aminotriazole produces unique physiological responses in plants, and because it is highly effective as a herbicide and defoliant at very low concentrations, development of an analytical method for studies of technical material and soil residues becomes important. A colorimetric analytical procedure based upon the reaction of aminotriazole with a modified sodium nitroprusside reagent has been developed and found applicable to these needs.

Extensive investigations have shown that the disappearance of aminotriazole is dependent on soil type and that the biological response of plants is proportional to the amount found in any soil type.

Determination of 3-Amino-1,2,4-triazole in Soils

Marston (6) found that guanidine gives a red color with a sodium nitroprusside reagent and used this test in his estimation of the chemical. The author discovered that small amounts of aminotriazole give a grassy green color with the same reagent. Moreover, the reaction conforms to Beer's law and as little as 1 p.p.m. of aminotriazole can be detected in aqueous solutions.

The sodium nitroprusside reagent used in ensuing studies is prepared as follows: sodium nitroprusside, $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$, 59.6 grams per liter; potassium ferrocyanide, $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$, 84.4 grams per liter; 10% sodium hydroxide; 3% hydrogen peroxide. The solutions are mixed together with constant stirring, in the proportions of 2:2:1:5; respectively, about 15 minutes before the reagent is used. As the optimum pH for greatest color development is between 10.8 and 11.2, 1.2 ml. of acetic acid is added per 100 ml. of the mixed solutions, bringing the pH to 11.0. The maximum absorption for the reaction between the nitroprusside reagent and aminotriazole occurs at 634 μ . A Fisher electrophotometer adapted with a 650 μ filter and a cell having a 23-mm. light path was used in all aminotriazole analyses.

In the preparation of a standard curve 0.200 gram of pure aminotriazole (melting point 157–158° C.) was dissolved in distilled water and diluted to 2 liters. Thus, 1 ml. contained 0.1 mg. of the

compound. Amounts from 0.1 to 1.6 mg. were delivered by a buret into 100-ml. volumetric flasks and diluted to about 70 ml. Next, 3 drops of 10% sodium hydroxide and 10 ml. of the nitroprusside reagent were added. After being diluted to the mark, the solutions were allowed to stand 45 minutes at room temperature. At the end of this time they were filtered, the color absorptions being measured 1 hour after the reagent was added. Reproducible results are obtained for these concentrations, if readings are taken within 5 minutes of the suggested time limit. If the concentration of aminotriazole is greater than 1.6 mg. per 100 ml., a longer time interval is necessary for completion of the color-producing reaction.

Technical grade material can be analyzed similarly, and best results are obtained when a standard is carried along simultaneously and calculations are based accordingly.

Experiments were conducted next to extract aminotriazole from a Maury loam soil from Kentucky. Attempts to recover all of the aminotriazole using 0.1N hydrochloric acid, 0.1N sodium hydroxide, 5% potassium sulfate, or distilled water were unsuccessful. This gave a hint that the compound was being adsorbed on the soil particles in the same manner as ammonium ion. For this reason the extraction of aminotriazole was studied using various salts, following Harper's procedure (4) for recovering ammonia from soils.

All solutions were prepared equivalent to the cation concentration of potassium in 5% potassium sulfate ($\text{K} = 0.575N$). The data summarized in Table I show that none of the salts used extracted more aminotriazole than did water alone. This indicated that the chemical was adsorbed on the soil particles very strongly, was held in some other chemical

Table I. Recovery of 3-Amino-1,2,4-triazole from a Maury Loam Soil Using Different Salt Solutions

(200 grams of soil and 300 ml. of extracting solution used)

Mg. 3-AT Theoretically Present in 2.5-Ml. Aliquot	Aminotriazole Found, Mg.							
	H ₂ O	K ₂ SO ₄	KCl	2 KCl	KNO ₃	KC ₂ H ₃ O ₂	NaCl	Na ₂ SO ₄
0.39	0.33	0.32	0.32	0.30	0.29	0.30	0.32	0.31
0.78	0.68	0.65	0.65	...	0.61	0.63	0.66	0.64
1.17	1.02	0.96	0.97	0.94	0.94	0.94	0.93	0.97

Table II. Mechanical Analysis, pH, Organic Matter, and Base Exchange of Several Soil Types

	Soil Types						
	Duke	Cecil	Croton	Maury	Yolo	Raub	Muck
Sand, %	91.2	53.8	39.8	44.8	66.4	34.8	...
Silt, %	6.8	37.4	51.2	45.4	18.4	49.4	...
Clay, %	2.0	8.8	9.0	9.8	15.2	15.8	...
pH	5.06	5.48	5.90	6.95	7.05	5.72	5.72
Organic matter, %	1.5	2.5	3.9	3.1	4.5	4.1	...
Exchange capacity, meq./100 g. soil	3.6	5.8	11.0	10.2	29.5	20.6	99.1

Table III. Disappearance of Aminotriazole in Various Soil Types

Soil Type	Days after Treatment							
	0	2	7	21	42	49	56	63
	Per Cent of Original Aminotriazole Present							
Duke	93.0	63.0	59.0	53.5	46.2	45.6	39.5	41.6
Cecil	63.2	58.5	52.4	43.6	35.8	33.6	27.7	28.0
Croton	74.3	68.0	58.2	47.9	38.7	35.2	35.8	35.8
Maury	79.1	65.6	57.0	43.5	34.7	34.7	29.2	29.2
Yolo	88.8	66.5	49.5	29.0	12.7	10.3	7.0	6.5
Raub	32.3	32.3	27.6	16.2	11.8	13.6	11.3	10.2
Muck	31.2	16.2	5.9	6 ^a	5 ^a	4 ^a	3 ^a	2 ^a

^a Estimated from observance of albinism on tomato seedlings; work done at Stamford Laboratories.

Table IV. Correlation of Aminotriazole Disappearance with Base Exchange of Soils

Group	Soil Type	Base Exchange, Meq./100 G. Soil	Clay Content, %	Days after Treatment					
				10	21	42	49	56	63
				Soil Rank with Respect to Most Aminotriazole Found					
A	Duke	3.6	2.0	1	1	1	1	1	1
B	Cecil	5.8	8.8	4	3	3	3	4	4
	Maury	10.2	9.8	3	4	4	4	3	3
	Croton	11.0	9.0	2	2	2	2	2	2
C	Raub	20.6	15.8	6	6	6	5	5	5
	Yolo	29.5	15.2	5	5	5	6	6	6
D	Muck	99.1	Not measured	7	7	7	7	7	7

combination, or both. Conclusive evidence that aminotriazole could be taken up in an ion exchange system was obtained when it was shown that a synthetic cation exchanger completely removed the compound from aqueous solutions.

As it was now apparent that aminotriazole could be adsorbed strongly on certain media, it was desirable to investigate the characteristics of some soil types, and to determine the rate of aminotriazole disappearance in them. It was hoped that a better understanding of the reactions in soils and their relationship to biological activity thus could be obtained.

Mechanical analyses were made on the following soil types according to Bouyoucos' procedure (7): Duke's sand from Long Island, Maury loam, Raub loam from Indiana, Yolo sandy loam from California, Cecil sandy loam from North Carolina, and Croton silty loam from New Jersey. Percentage of organic matter was determined on the same soil types by the method of Schollenberger (77). The exchange capacity based on the dry weight of each soil type was determined accordingly by the method of Peech (9). Measurements of pH were also made. These data are compiled in Table II.

The studies of aminotriazole dis-

appearance in the soils were carried out as follows: An appropriate amount of the chemical was mixed with 4000 grams of air-dried soil to give 60 p.p.m. of aminotriazole on an oven-dry weight basis. Mixing was done by a rolling machine for 20 minutes. Next, sufficient water was added to bring each soil type to 20% of its moisture-holding capacity. The soils were then placed in 1-gallon wide-mouthed glass jars, capped, and stored in a constant temperature room at 68° F. Aminotriazole was determined colorimetrically on 100-gram samples at different intervals.

The amount of water used to extract the soil samples varied from 100 to 250 ml., and the aliquot was either 25 or 50 ml. Reagent blanks were run on untreated portions of each soil type. Samples for analyses were taken after the soil slurries had been vigorously agitated for 15 minutes. This time was set arbitrarily, because it was found that no additional aminotriazole was released from the soil for a longer extraction period, even up to 5 hours.

The data given in Table III and plotted in Figure 1 are interesting. At the end of 9 weeks practically all the aminotriazole had disappeared from the muck soils. The Yolo and Raub soils had only a little more present, 4 and 6 p.p.m., respectively. The Cecil and Maury soils were next with 17 and 19 p.p.m. of aminotriazole, while the Croton and Duke soils contained 23 and 26 p.p.m.

When the percentage of original chemical remaining was calculated for each soil, a strong correlation was found between aminotriazole disappearance and base exchange capacity. As indicated in Table IV, the soil types may be separated into four groups according to their exchange capacities and clay content. When the order of least aminotriazole disappearance is then ranked for each soil type from about the tenth day until the end of the experiment, it is found to be almost in direct proportion to the magnitude of the respective base exchanges and clay content. The muck soil, which has the highest exchange capacity, was the soil from which the chemical disappeared fastest; next were Raub and Yolo, Cecil, Maury, and Croton, and finally Duke's sand.

It seems that the various soil types arrive at an equilibrium reaction in an exchange complex with aminotriazole.

Table V. Tomato Seedling Response to Aminotriazole Toxicity

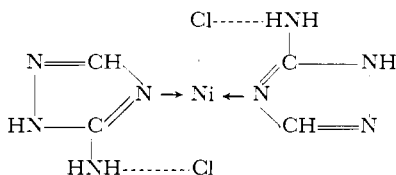
Expt. No.	3-AT Found at Planting Time, P.P.M.							Days after Seedlings Planted	Observed Plant Response							
	Duke	Croton	Maury	Cecil	Raub	Yolo	Muck		Duke	Croton	Maury	Cecil	Raub	Yolo	Muck	
1	58	48	50	39	20	55	20	33	A	A	A	A	A	A	A	
2	39	43	42	36	20	41	10	21	A	A	A	A	A	A	A	
3	37	37	36	32	17	30	8	26	A	A	A	A	A	A	A	
4	33	31	28	27	10	18	6	12	A	A	A	A	A	A	D	
5	29	25	22	22	7	8	4	8	A	B	B	B	D	D	E	
6	28	23	22	21	8	6	3	15	A	A	A	A	A	A	A	
								12	A	A	A	A	A	A	F	F
								16	A	A	A	A	A	A	D	C
7	25	23	19	17	7	4	2	5	F	E	E	F	G	G	H	
								9	B	D	C	E	E	F	G	
								12	A	A	A	A	A	A	F	F
8	26	23	19	17	6	4	1	14	A	A	A	A	A	D		
								2	G	F	F	G	H	I	I	
								5	F	E	E	F	G	G	H	
								7	E	D	C	F	F	F	H	

- A. Dead
- B. Severe necrosis
- C. Complete albinism of true leaves
- D. Severe albinism of true leaves
- E. Albinism in true leaves, cotyledons yellowish and not turgid
- F. Albinism in true leaves but cotyledons turgid
- G. Albinism in various degrees and cotyledons normal
- H. Very little albinism
- I. Normal

Soils with a high base exchange but a low degree of cation saturation would be expected to adsorb relatively large amounts of aminotriazole rapidly. The variances obtained in the initial analyses of the various soil types are probably due to different degrees of cation saturation among the soils. The complexing of adsorbed cations or insoluble cations followed by adsorption of the aminotriazole metal complex molecules thus formed may be another factor.

Studies in these laboratories have shown that aminotriazole possesses the ability to form stable complexes with several metals, including cobalt, copper, nickel, iron, and magnesium. The

nickel complex was found to have the following structure:



Infrared examination of 3-amino-1,2,4-triazole and the nickel complex showed that although the compounds were different, a strong absorption for the amino group was evident in both cases. Thus, it seems possible that even the metal complexes can enter into the

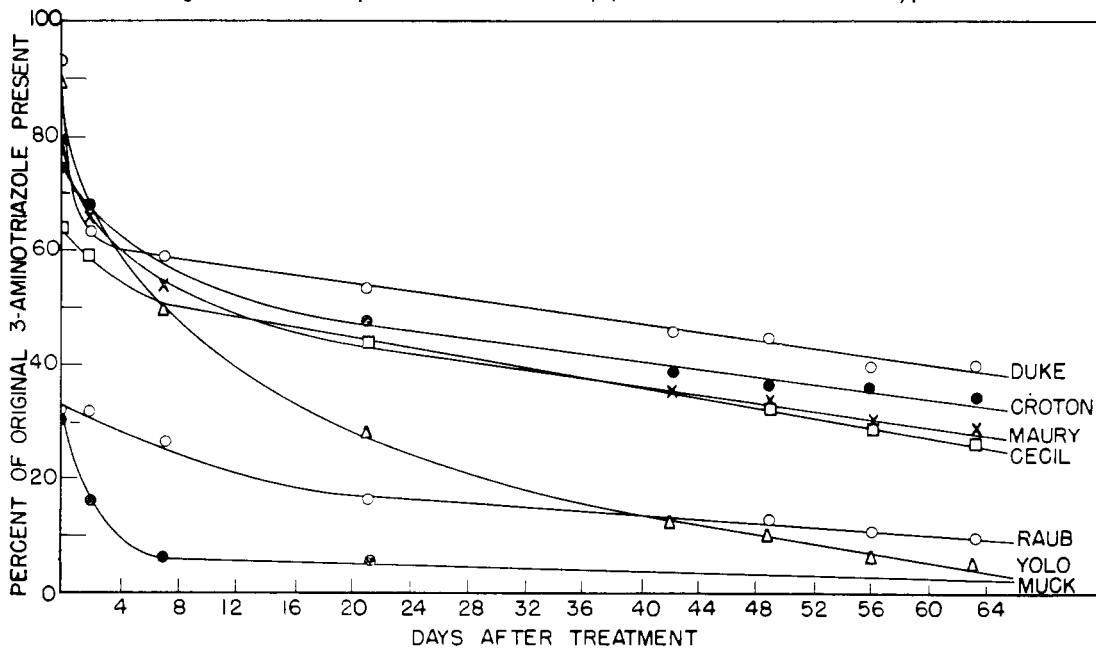
soil exchange system. As the Yolo soil is high in calcium and magnesium, the disappearance of aminotriazole from this soil type is probably due to a combination of complexing and adsorption effects.

The ability of aminotriazole to form complexes with metals, along with its similarity to the pyrrole structure, indicates that precursors which may enter into the synthesis of pseudo-chlorophyllous porphyrins are possible. That aminotriazole may replace one or more pyrrole groups in the synthesis of chlorophyll by the plant has been recognized independently by other workers (3).

The experiments on residual activity of aminotriazole do not necessarily show

that the chemical has long residual effects under field conditions. Rather, cognizance must be taken of the fact that less aminotriazole should be required for soil treatment work when the base exchange capacity is low than when the soil is heavy and contains much organic matter. The data obtained would seem to indicate why such workers as Ny-lund (8), in his experiments on pre-emergence weed control in muck soils, did not find aminotriazole as effective as other herbicides in this particular soil type.

Figure 1. Decomposition of 3-amino-1,2,4-triazole in various soil types



Physiological Response of Tomato Seedlings to Aminotriazole Toxicity

In order to evaluate the reliability of the determination of aminotriazole in soils, biological observations were made on tomato seedlings planted concurrent with chemical analyses. Small clay pots were used and the plants were sub-irrigated. In practically all instances the amount of aminotriazole found by chemical analyses was proportional to the degree of plant injury. Table V gives comparisons between the amount found and the plant responses for each soil type.

Physiological reactions were concordant with the amounts of aminotriazole found in all soils, although the recoveries of aminotriazole varied.

Thus, the quantity of chemical that was extractable from the soil definitely appeared to correlate with the amount the plant itself was able to absorb. Observations on aminotriazole toxicity to tomato seedlings 8 days after planting indicated that complete albinism in the true leaves to severe necrosis occurred when as much as 25 p.p.m. of chemical was present. At a concentration of 20 p.p.m. severe albinism was noted, while 5 to 7 p.p.m. of aminotriazole caused albinism in various degrees with loss of turgidity in the cotyledons. These leaves generally do not turn white but change to various shades of yellow when affected. When only 2 p.p.m. of aminotriazole was present, albinism

occurred in 8 days with little change in the cotyledons.

Further observation showed the degree in which aminotriazole affected tomato seedlings from 2 to 12 days after planting. When as much as 27 p.p.m. of chemical was present, albinism occurred in 2 days, became more widespread in 5 days, then developed severe necrosis, followed by death of the plant in 8 to 12 days. At a concentration of 7 p.p.m. albinism was evident in 2 to 5 days and became severe at 8 days. When only 2 p.p.m. of aminotriazole was present, albinism was noted at 5 days but was more pronounced one week later.

It appears that knowledge of a soil's base exchange capacity can definitely aid in planning pre-emergence work. The unique structural, adsorption, and metal-complexing properties and the effect of 3-amino-1,2,4-triazole on plant processes offer unusual possibilities for basic research in plant physiology.

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FERTILIZER GRANULATION

Effects of Formulation on Granulation of Mixed Fertilizers

MORE THAN 80 of some 1200 fertilizer mixing plants in the United States are producing granular mixed fertilizers and the number of plants going into production is continuously increasing. It was estimated that, at the close of 1955, the capacity for annual production of granular mixtures in this country exceeded 2,000,000 tons, or 12% of the total production of mixtures. This trend toward greater production of granular mixed fertilizers is accompanied by the need for basic information on optimum conditions for agglomeration of a wide range of formulations.

The volume of liquid phase present at the surface of solid particles during the processing of a mixed fertilizer is one

of the most important factors governing the degree of agglomeration (1, 5). The liquid phase consists of an aqueous solution of the soluble constituents of the mixture. Its volume is dependent on the types and amounts of salts in the liquid phase of the mixture as well as on the moisture content and temperature (7-4). This volume of liquid phase is not readily determined by direct means (7), but the agglomeration characteristics of mixed fertilizers can be ascertained by correlating the soluble salt content of the mixture with the moisture requirement for optimum agglomeration at a given temperature. Accordingly, the present paper relates to a study of the moisture requirement

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for optimum agglomeration of mixed fertilizers formulated with triple and ordinary superphosphates, anhydrous ammonia, and varying proportions of ammonium nitrate, ammonium sulfate, and potassium chloride alone and in combination. The data should serve as a guide in predicting the relative effect of formulation on moisture requirement for optimum agglomeration of mixed fertilizers under fixed conditions of plant operation. This study is part of a broad research program on the preparation of high-analysis fertilizers of improved physical condition being conducted in cooperation with the Tennessee Valley Authority.